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Inactivation kinetics and photoreactivation of vegetable oxidative enzymes after combined UV-C and thermal processing☆

Fernando Sampedro [a,b], Xuetong Fan [b,*]

[a] Center for Animal Health and Food Safety, Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, 1354 Eckles Avenue, St Paul, MN 55108, USA
[b] Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA

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ABSTRACT

The inactivation kinetics of lipoxygenase (LOX), peroxidase (POD) and polyphenoloxidase (PPO) in phosphate buffer (pH 4.0 and 7.0) treated by combined thermal (25–65 °C) and UV-C (1–10 min) processes were fitted using a traditional first-order kinetics model and the Weibull distribution function. For complete inactivation, a treatment at 65 °C for 7.5–10 min for LOX, POD and PPO at pH 7.0 and 45 °C for 5–7.5 min for POD and PPO at pH 4.0 was necessary. Deviations from the log-linear behavior were observed by the appearance of shoulders, tails or both (sigmoidal). The traditional log-linear model failed to characterize the UV treatment effectively due to the under- and overestimation of enzyme inactivation. The Weibull model was better able to explain the nature of the UV treatment. The extent of enzyme inactivation was less in orange juice due to the greater absorbance of the juice in the UV-C range. In general, activities of residual enzymes after UV-C treatment did not recover after storage for 24 h at refrigeration conditions with or without light exposure. The proposed combination of thermal and UV-C processing was able to improve the stability of the treated samples.

Industrial relevance: UV irradiation has demonstrated to be an effective technology to decontaminate surfaces and reduce microbial load of liquid food in a low cost, simple and chemical-free manner. However, its application in the pasteurization of liquid food needs to be validated against achieving an adequate enzymatic stability. A kinetic study on the inactivation of quality-related enzymes (peroxidase, lipoxygenase and polyphenoloxidase) after the combined thermal and UV-C processing (25–65 °C for 1–10 min) was conducted. The combined treatment (45–65 °C for 5 min) was able to achieve a complete reduction of enzymatic activity. The extent of enzyme inactivation was less in orange juice due to the greater absorbance of the matrix. Enzymes were irreversibly inactivated and enzyme activities did not recover after storage.

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1. Introduction

Ultraviolet (UV) light irradiation has been used for decades to disinfect water, solid surfaces and packaging containers in the food industry (Koutchma, Forney, & Moraru, 2009). The germicidal effects of UV irradiation reside on preventing the microorganisms from reproducing by disrupting their nucleic acid. The effectiveness of UV light is closely related to the UV light absorption capacity of nucleic acids which occurs at an optimal wavelength of 254 nm (also called the 'germicidal wavelength', UV-C generated by low-pressure mercury lamps) (Koutchma, 2009). Research efforts lately have been focused on the application of UV irradiation for the pasteurization of liquid foods such as fruit juices, apple cider, milk and liquid egg (Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004; Geveke, 2008; Matak et al., 2004; Oteiza, Peltzer, Gannuzzi, & Zaritzky, 2005). UV irradiation has shown the potential to inactivate pathogen and spoilage microorganisms in a low cost, simple and chemical-free manner (Guerrero-Beltran & Barbosa-Canovas, 2004). These research developments led the U.S. Food and Drug Administration (FDA) to release a pre-market approval (21 CFR 179.39) for the treatment of water and foods with UV-C technology (U.S. Food and Drug Administration, 2004).

Besides food safety, food quality is another important aspect when designing a new food preservation method. Oxidative enzymes such as lipoxygenase (LOX), peroxidase (POD) and polyphenoloxidase (PPO) are responsible for detrimental quality effects in fruit juices such as enzymatic browning, lipid oxidation, pigment bleaching and off-flavor development, inducing changes in flavor, color and nutritional value (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2002). Few studies are available on the effects of UV-C on enzymes in fruit juices. Some

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* Corresponding author. Tel.: +1 215 836 3753; fax: +1 215 233 6581.
E-mail address: xuetong.fan@ars.usda.gov (X. Fan).

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studies (Noci et al., 2008; Tran & Farid, 2004) showed negligible effects of UV-C processing on pectinmethylesterase in orange juice and POD and PPO in apple juice, whereas Falguera, Pagan, and Ibarz (2011) demonstrated complete inactivation of POD and PPO in apple juice after long UV exposure times (15 and 100 min, respectively). These contradictory results seem to reflect a lack of understanding about the effects of UV-C processing on enzymes and the need for more comprehensive studies.

The combination of thermal and nonthermal processing has been widely shown to have a synergistic effect on enzyme inactivation and to be an energy saving strategy when combining elevated temperature with pressure (Ludikhuyze, Van Loey, Indrawati, Smout, & Hendrickx, 2003) and pulsed electric fields (Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003). However, there are no studies in the scientific literature on the enzyme inactivation kinetics in response to combined thermal and UV-C processing. Most often, data on enzyme inactivation kinetics are explained by the use of first-order kinetic models that assume a linear relationship between the logarithm of enzyme activity and exposure time. This is usually not true, and deviations from the log-linear behavior such as the appearance of a lag phase (shoulder), a tail, or both (sigmoidal) or a combination of linear and nonlinear behaviors are often observed (Huang, Tian, Gai, & Wang, 2012). The use of kinetic models that accurately describe the nonlinearity of enzymatic kinetic curves is crucial.

One of the main limitations when designing an UV irradiation treatment is the presence of dissolved organic solutes and compounds such as sugars and organic acids and suspended solids that absorb and scatter UV light, consequently greatly reducing the germicidal effect of UV (Koutchma, 2009). Other potential limitations are photoreactivation and dark repair effects which are defined as the ability to repair the UV-damaged nucleic acid after processing by means of light exposure or in the absence of light (Oguma et al., 2001). Enzymes are, in some cases, able to reconfigure their structure when reversible damage has occurred during processing, allowing them to totally or partially recover their initial activity during storage. Such is the case with POD after high pressure processing (Anese, Nicoli, Dall'Aglio, & Lerici, 1995), POD after blanching treatment (Schweiggert, Schieber, & Carle, 2006), and PPO after super critical carbon dioxide processing (Gui et al., 2006). The potential for enzyme repair after UV processing is unknown and might be detrimental to the food quality of UV-treated products, shortening their shelf-life.

The objectives of this study are to characterize the combined thermal and UV-C inactivation kinetics of LOX, POD and PPO enzymes and to investigate the effects of pH, absorbance and photoreactivation.

2. Material and methods

2.1. Enzyme samples and activity measurements

Lipoxygenase (LOX, EC 1.13.11.12) from soybean with 158,000 units/mg, peroxidase (POD, EC 1.11.1.7) from horseradish with 256 units/mg, and polyphenoloxidase (PPO, EC 1.14.18.1) from mushroom with 98,800 units/mg were obtained from Sigma-Aldrich (St. Louis, MO). Enzymes were dissolved in sodium phosphate buffer (0.2 M, pH 7.0) and sodium acetate buffer (0.2 M, pH 4.0) at concentrations close to those naturally found in food: 50 μg/mL (LOX), 20 μg/mL (POD) and 35 μg/mL (PPO). All the chemicals used for the enzymatic analysis were also obtained from Sigma-Aldrich.

Enzyme measurement protocols for LOX, POD and PPO can be found in a previous manuscript by the same authors (Sampedro, Phillips, & Fan, 2013). LOX activity was measured according to a method described by Rodrigo, Jolie, Van Loey, and Hendrickx (2007) and Aguiló-Aguayo, Sobrino-López, Soliva-Fortuny, and Martín-Belloso (2008) with slight modifications by using linoleic acid as the substrate and measuring absorbance at 234 nm every second for 300 s. POD assay was based on a procedure described by Cano, Hernandez, and DeAncos (1997) and Garcia-Palazon, Suthanthangjai, Kajda, and Zabetakis (2004) with slight modifications by using *p*-phenylenediamine as a substrate. Absorbance was recorded at 485 nm every second for 90 s. PPO activity was based on the procedure described by Weemaes et al. (1997) with some modifications by using catechol as the substrate. The change of absorbance was recorded at 411 nm for 90 s. For all the enzyme measurements, the slope of the linear part of the curve was determined and one unit of enzyme was defined as the change in absorbance per minute at 25 °C.

2.2. Physical measurements

Absorbance and transmittance were measured using a UV spectrophotometer (Shimadzu UV-2401PC, Shimadzu Scientific Instruments Inc. Columbia, MD). Absorption coefficient (α) is a physical measure used to compare the UV absorbance of liquid foods and is defined as the absorbance divided by the path length (cm^{-1}) and was calculated as follows (Koutchma et al., 2009):

$$\alpha = 2.303A_{254} \tag{1}$$

where α is the absorption coefficient (cm^{-1}) and A_{254} is the absorbance at 254 nm. pH was measured by means of a pH-meter (Orion 420A+, Thermo Electron Corp., Beverly, MA). Measurements were made in triplicate, and average values were reported with standard deviations.

2.3. UV-C treatment

A detailed description of the UV-C treatment was published previously (Sampedro et al., 2013). Briefly, the UV-C treatment was conducted using two ultraviolet low-pressure mercury irradiator bulbs with maximum emissions at 254 nm (Atlantic UltraViolet Corp., Hauppauge, NY). Fifteen milliliters of enzyme sample was distributed as a thin layer in a glass dish and placed on a stirring plate with a flexible silicone rubber heater attached (Omega Engineering Inc., Stamford, CT). A PID controller (Model CN4431, Omega Engineering Inc) was used to maintain a constant sample temperature at selected temperatures between 25 and 65 °C during the process by supplying energy to the heater. A thermocouple (K type) in contact with the sample was used to monitor temperature. Samples were irradiated for different exposure times (1–10 min) and collected in screw-cap glass tubes, wrapped with aluminum foil to avoid photoreactivation, and immediately immersed in an ice-water bath until the analysis of enzyme activity. Enzyme activity of control (untreated) (A_0) and UV-treated samples was measured (A). Treatments and enzyme activity determinations were conducted in triplicate.

2.4. Mathematical models

The effects of the thermal and UV-C inactivation kinetics of LOX, POD and POD in the sodium acetate buffer at pH 4.0 and sodium phosphate buffer at pH 7.0 were described using the traditional first-order kinetics model. Next to characterization in terms of inactivation rate constants (k) (Eq. (2)), first-order inactivation kinetics were described by decimal reduction times, D-values [time needed to reduce the initial activity by one log unit (90%) at a constant temperature] (Eq. (3)):

$$\mathrm{Log}(A) = \mathrm{Log}(A_0) - kt \tag{2}$$

$$\mathrm{Log}(A) = \mathrm{Log}(A_0) - \frac{t}{D} \tag{3}$$

where A is the dependent variable and can be expressed as enzyme activity (U/mL), A_0 is the initial enzyme activity (U/mL), k is the inactivation rate constant (min^{-1}), D is the decimal reduction time (min) and t is the independent variable expressed as the UV exposure time (min). The k and D parameters were estimated through a linear regression.

Residual enzyme activity curves following UV processing have shown deviations from the log-linear behavior by the appearance of

an initial lag or tailing phase or both (Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004; Sastry, Datta, & Worobo, 2000). The Weibull distribution function has been used in several studies (Elez-Martinez, Suarez-Recio, & Martin-Belloso, 2007; Lemos, Oliveira, Van Loey, & Hendrickx, 1999; Rodrigo, Barbosa-Canovas, Martinez, & Rodrigo, 2003) to characterize the enzyme inactivation kinetics after nonthermal processing and was used to fit experimental data by using the following equation:

$$A = A_0 \exp\left(-\frac{t}{\alpha}\right)^{\beta} \quad (4)$$

where A and A_0 have the same meaning as in Eqs. (2) and (3) (U/mL), t is the independent variable as the UV exposure time (min), α is the time required for 90% enzyme inactivation (min), and β is the shape parameter. The β value gives an idea of the form of the curve, if $\beta > 1$ the curve is convex (it forms shoulders), if $\beta < 1$ the curve is concave (it forms tails) and if $\beta = 1$ the curve is a straight line and can be described by linear models. The parameters (α and β) were estimated through a nonlinear regression.

Two replicates were used to establish how well the model fitted to the experimental enzyme inactivation curve by obtaining the values of the adjusted R^2 (Adj. R^2) defined as follows:

$$\text{Adj.R}^2 = \left[1 - \frac{(m-1)\left(1 - \frac{SSQ_{regression}}{SSQ_{total}}\right)}{m-j}\right] \quad (5)$$

where m is the number of observations, j is the number of model parameters, and SSQ is the sum of squares.

The predictive models were then validated with a third set of experimental data that was not used for model fitting, by calculating the mean square error (MSE), defined as follows:

$$\text{MSE} = \frac{\sum(\text{predicted} - \text{observed})^2}{n-p} \quad 6$$

where n is the number of observations, the predicted and observed values refer to predicted and observed enzyme activities, respectively, and p is the number of parameters estimated by the model. The smaller the MSE values, the better the model fits the data.

2.5. Orange juice

Orange juice without pulp (Florida's Natural Brand Original, Florida's Natural, Lake Wales, FL) was purchased from a local supermarket and stored at 4 °C. Orange juice samples were centrifuged at 16,000 *g* for 20 min at 4 °C using a Sorvall RT6000B refrigerated centrifuge (DuPont Co. Wilmington, Del.), and enzymes were dissolved in the juice supernatants according to the above section. Enzyme samples were UV-C treated at 25 and 65 °C for 1 and 10 min in the sodium acetate buffer (pH 4.0) and orange juice. Measurements were done in duplicate, and average values were reported with standard deviations.

2.6. Photoreactivation

Enzyme samples were UV-C treated at 25 and 65 °C for 1 and 10 min at pH 4.0 and 7.0, and then aliquots of 20 mL were placed in screw-cap glass tubes (foil wrapped and unwrapped) for 24 h at 4 °C in an incubator under constant illumination by one horizontally fixed fluorescent lamp (GE F17T8-SP41, 17 W with average 1260 lm). Initial and final enzyme activity after 24 h was measured to show changes in enzyme activity. Measurements were done in duplicate, and average values were reported with standard deviations.

3. Results and discussion

3.1. Enzyme inactivation kinetics after combined thermal and UV-C processing

The inactivation kinetic curves for LOX, POD and PPO enzymes in phosphate buffer (pH 4.0 and 7.0) obtained after the combined thermal and UV processing (25–65 °C and 1–10 min) showed that higher UV exposure time and temperature favored the inactivation (Fig. 1). Residual activity values of 0.15 (85% inactivation) in LOX activity were achieved after UV treatment at 65 °C for 7.5 min at both pH values. Complete POD inactivation was observed at 45 and 65 °C for 7.5 and 2.5 min, respectively, at pH 4.0, and at 65 °C for 10 min at pH 7.0. Residual PPO activity of 0.10 (90% inactivation) was obtained at 45 and 65 °C for 5 and 1 min, respectively, at pH 4.0, and complete inactivation was obtained at 65 °C for 7.5 min at pH 7.0. Elevated temperature is known to affect enzyme activity by inducing protein aggregation and denaturation.

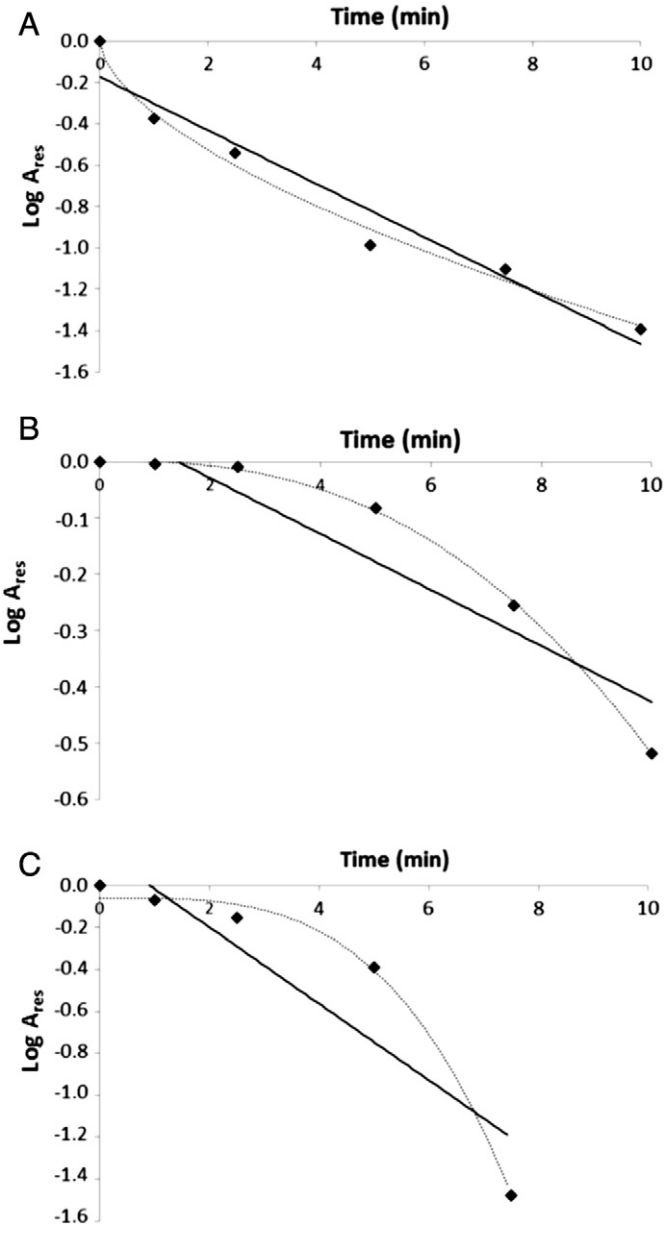

Fig. 1. Inactivation kinetics curves of PPO at pH 4.0 and 45 °C (A), LOX at pH 7.0 and 25 °C (B), and POD at pH 7.0 and 65 °C (C). Solid line represents the linear model fitting. Dotted line represents the Weibull model fitting.

Table 1
Physical measurements at 254 nm.

Enzyme	Phosphate buffer pH 4.0		Phosphate buffer pH 7		Orange juice	
	Absorption coefficient (α_{10}) (cm^{-1})	Transmittance (%)	Absorption coefficient (α_{10}) (cm^{-1})	Transmittance (%)	Absorption coefficient (α_{10}) (cm^{-1})	Transmittance (%)
LOX	0.19	82.45	0.28	78.22	9.21	0.022
POD	0.15	85.78	0.32	73.65	9.45	0.022
PPO	0.22	80.34	0.24	80.71	9.50	0.020

Table 2
Log-linear and Weibull inactivation kinetic parameters and standard error describing the combined thermal and UV-C inactivation of LOX.

T (°C)	pH	Log-linear			Weibull			
		k (min^{-1})	Adj. R^2	MSE	α (min)	β	Adj. R^2	MSE
25 °C	4.0	$0.05 \pm 0.01^{a*}$	0.84	0.001	$22.06 \pm 6.64^{a*}$	1.96	0.91	0.000
45 °C		0.08 ± 0.01^{a}	0.84	0.003	$66.86 \pm 38.60^{a*}$	0.53	0.92	0.001
65 °C		0.19 ± 0.03^{b}	0.87	0.015	17.62 ± 10.73^{a}	0.43	0.75	0.024
25 °C	7.0	$0.11 \pm 0.02^{a*}$	0.84	0.007	$12.91 \pm 0.13^{a*}$	2.54	0.99	0.000
45 °C		0.06 ± 0.01^{b}	0.91	0.001	$20.28 \pm 3.96^{b*}$	1.84	0.95	0.000
65 °C		0.16 ± 0.02^{c}	0.90	0.007	$15.34 \pm 3.63^{a,b}$	0.80	0.88	0.009

[a, b, c]: Different letters indicate statistical difference due to temperatures ($p < 0.05$).
* Statistical difference due to pH values ($p < 0.05$).

Treatment at 25 and 35 °C for 10 min at either pH 4 or 7 had no effect on the enzyme activities, whereas treatment at 45 and 55 °C had little effect on the enzyme activities (4–18% reduction) suggesting that inactivation at that temperature range was achieved mainly by UV radiation. At 65 °C the impact of temperature on enzyme activity was variable due to the different exposure times with inactivation rates from 18 to 66% for LOX, 10 to 64% for POD and 22 to 89% for PPO. The combination of UV and thermal processing at 65 °C enhanced the inactivation by 8–36% for LOX, 5–64% for POD and 2–20% for PPO. Several authors have shown that UV-C processing has no effect on PME in orange juice (Tran & Farid, 2004) and POD and PPO in apple juice (Noci et al., 2008). On the other hand, Falguera et al. (2011) showed complete POD and PPO inactivation after UV-C treatment for 15 and 100 min, respectively, using a batch system. The data obtained in our study demonstrate that moderately-high temperatures in combination with UV-C irradiation represent an optimal strategy for reducing the enzymatic activity.

Data for the combined thermal and UV-C inactivation trials were fitted to the traditional log-linear model and the Weibull distribution function (Eqs. (2), (3) and (4)). These resulted in estimates of the rate constants (k and α) for each of the enzymes (Tables 2, 3 and 4). D values (min) were estimated according to Eq. (3) and values ranged from 46.1 to 12.1 min for LOX, 19.2 to 4.2 min for POD and 115.2 to 4.7 min for PPO. Estimated D values for POD were the lowest among the three enzymes, indicating that POD was the most UV-labile enzyme. D values of PPO in mango nectar after UV-C treatment ranged from 152 to 199 min according to Guerrero-Beltran and Barbosa-Canovass (2006). The D values reported in our study were significantly lower than those reported in the mango study as a result of the higher destruction rate of the combination of UV-C treatment and elevated temperature.

The majority (61%) of the inactivation kinetic curves presented deviations from the log-linear behavior, forming pronounced shoulders and tails (downward and upward concavity) (Fig. 1). This is an indication that more than one fraction (isoenzyme) is present with varied resistance to the treatment (labile and resistant fractions). The presence of a number of fractions is not new, and several authors have reported them in LOX (Anese & Sovrano, 2006), POD (Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002) and PPO (Weemaes et al., 1997). These deviations from log-linear behavior impacted the goodness of fit values (Adj. R^2 values) (0.39 to 0.98) and prediction errors (MSE values) (0.02 to 61%) for the log-linear model for which the predictions were less accurate than those for the Weibull model (Adj. R^2 values of 0.70–0.99 and MSE values between 0.01% and 42%) (Tables 2–4). The nonlinear behavior observed in the kinetic curves could also lead to a miscalculation of the optimized conditions for UV-C pasteurization when using D values due to the under- and overestimation of enzyme inactivation. For these reasons (better goodness of fit and more accurate characterization of treatment effectiveness), the Weibull model and its kinetic parameter (α) were chosen to characterize the UV-C inactivation of enzymes and used for comparison purposes.

Values of the kinetic parameter (α) were significantly lower at pH 4.0 than pH 7.0 and at higher temperatures for all the treatment conditions indicating higher UV-C enzyme inactivation at acidic conditions

Table 3
Log-linear and Weibull inactivation kinetic parameters and standard error describing the combined thermal and UV-C inactivation of POD.

T (°C)	pH	Log-linear			Weibull			
		k (min^{-1})	Adj. R^2	MSE	α (min)	β	Adj. R^2	MSE
25 °C	4.0	$0.18 \pm 0.01^{a*}$	0.98	0.002	$12.25 \pm 0.26^{a*}$	1.39	0.99	0.000
45 °C		$0.51 \pm 0.06^{b*}$	0.94	0.043	$5.05 \pm 0.47^{b*}$	1.30	0.94	0.043
65 °C		0.55 ± 0.21^{b}	0.55	0.619	$0.68 \pm 0.23^{c*}$	0.39	0.70	0.416
25 °C	7.0	$0.12 \pm 0.01^{a*}$	0.95	0.002	$14.93 \pm 1.05^{a*}$	1.55	0.98	0.000
45 °C		$0.12 \pm 0.01^{a*}$	0.97	0.001	$16.43 \pm 0.95^{a*}$	1.39	0.99	0.000
65 °C		0.42 ± 0.11^{b}	0.77	0.084	$6.79 \pm 0.15^{b*}$	3.48	0.99	0.004

[a, b, c]: Different letters indicate statistical difference due to temperatures ($p < 0.05$).
* Statistical difference due to pH values ($p < 0.05$).

Table 4
Log-linear and Weibull inactivation kinetic parameters and standard error describing the combined thermal and UV-C inactivation of PPO.

T (°C)	pH	Log-linear			Weibull			
		k (min^{-1})	Adj. R^2	MSE	α (min)	β	Adj. R^2	MSE
25 °C	4.0	$0.14 \pm 0.01^{a*}$	0.95	0.003	$14.89 \pm 1.77^{a*}$	1.24	0.95	0.003
45 °C		$0.30 \pm 0.04^{b*}$	0.93	0.018	$5.88 \pm 0.78^{b*}$	0.60	0.98	0.005
65 °C		$0.44 \pm 0.21c$	0.39	0.656	$0.18 \pm 0.21^{c*}$	0.24	0.75	0.268
25 °C	7.0	$0.02 \pm 0.00^{a*}$	0.85	0.000	$26.89 \pm 7.46^{a*}$	2.37	0.94	0.000
45 °C		$0.08 \pm 0.01^{b*}$	0.89	0.002	$19.10 \pm 4.13^{b*}$	1.70	0.92	0.001
65 °C		0.49 ± 0.11^{c}	0.78	0.184	$5.92 \pm 2.31^{c*}$	1.42	0.74	0.218

[a, b, c]: Different letters indicate statistical difference due to temperatures ($p < 0.05$).
* Statistical difference due to pH values ($p < 0.05$).

and in combination with higher temperature ($p < 0.05$). However, no statistical differences were observed among the α values for LOX at different temperatures ($p > 0.05$) for both pH values indicating that the enzyme is stable to temperature changes. With increasing temperature, values of the shape parameter (β) decreased and the shape of the curve changed from pronounced shoulder to tail. This could be explained by the coexistence of labile and resistant fractions, and during mild treatment conditions (room temperature and short exposure times) no effect was produced on enzyme activity, while with increasing treatment intensity (exposure time and/or temperature), the labile fraction was inactivated leaving the residual resistant fraction active.

3.2. Orange juice serum

Fig. 2 shows the effects of UV-C processing (25 and 65 °C for 1 and 10 min) on enzymes dissolved in orange juice (pH 3.8) and acetic buffer (pH 4.0). Inactivation was significantly reduced in orange juice where inactivation percentages ranged from 6 to 11, 0 to 69 and 43 to 99% versus 14 to 75, 29 to 99, and 24 to 99% in buffer for LOX, POD and PPO, respectively. This is in agreement with Tran and Farid (2004) where the authors observed no effect on PME in orange juice after UV-C processing at room temperature. As observed earlier in this study, enzyme inactivation was enhanced by increasing the temperature and UV exposure time. LOX was found to be the most UV-resistant enzyme, and little treatment effect was found in orange juice, whereas PPO was completely inactivated at 65 °C for 10 min. In comparison with the previous results from this study, it seems that under acidic conditions, LOX enzyme is much more resistant and PPO is much more sensitive to UV-C irradiation. UV absorbance by liquids has been identified earlier as a critical factor for UV-C processing. Pigments, organic solutes (sugars and organic acids) and suspended matter of fruit juices increase the absorption and reduce the transmission of UV light, thereby lowering the performance efficiency of UV-C pasteurization (Koutchma et al., 2009). As shown in Table 1, orange juice sample had 15× fold higher absorptivity and 3000× fold lower transmittance values than buffer solutions. Different authors have studied the effect of liquid absorbance on the overall effectiveness of UV-C processing. Koutchma, Keller, Chirtel, and Parisi (2004, 2007) studied the inactivation of *Escherichia coli* K-12 in model solutions at different absorption coefficients, observing lower microbial destruction with increasing absorbance. As stated by the authors, in the case of juices with higher absorptivity, multiple passes may be required for the adequate stabilization of juices by UV-C processing. This treatment strategy may increase the processing costs. In our study, we showed that it is possible to reduce the POD and PPO enzymatic activity of orange juice by means of a combination of temperature and UV-C irradiation.

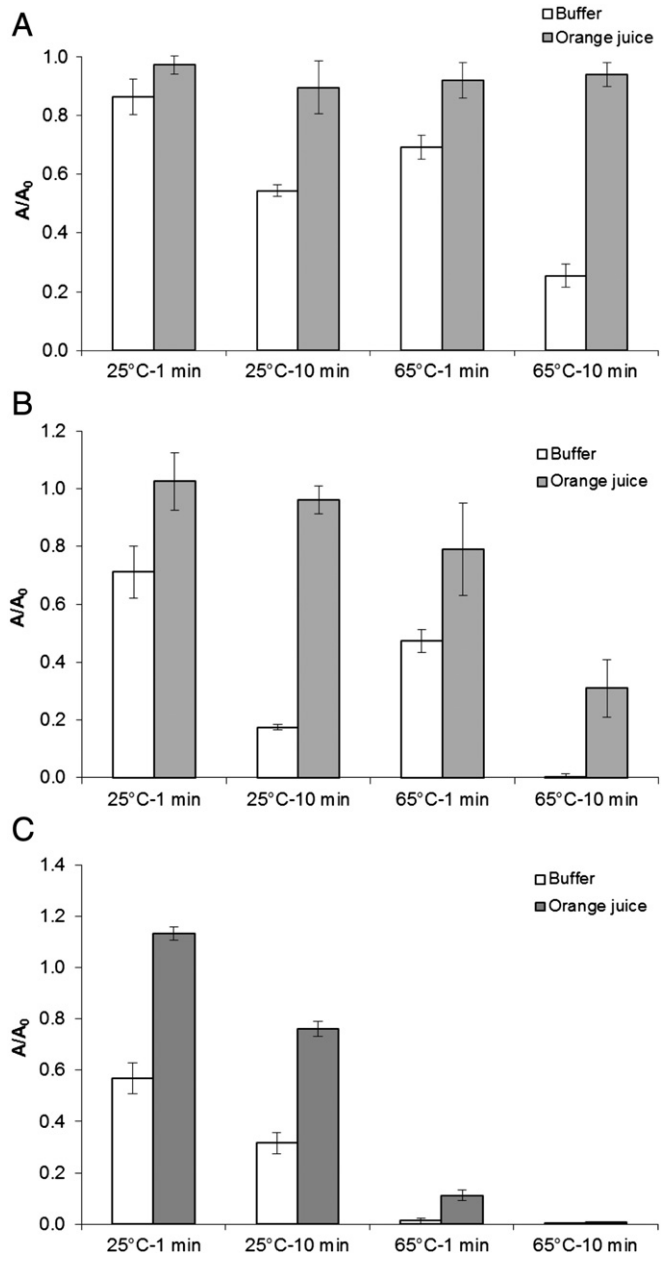

Fig. 2. Enzyme inactivation in acetic buffer (pH 4.0) and orange juice (pH 3.8). LOX (A), POD (B), PPO (C). Vertical lines represent standard deviation.

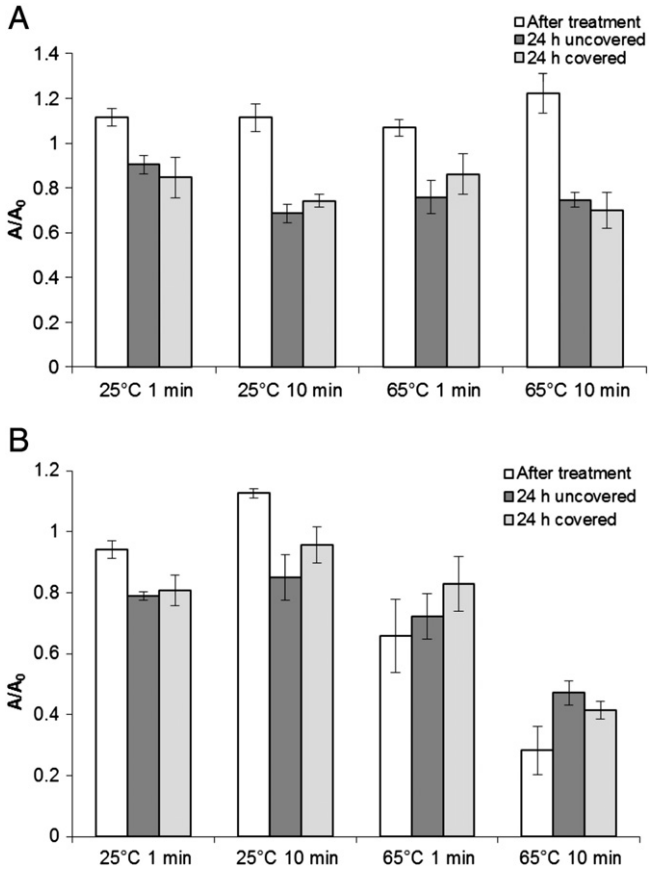

Fig. 3. LOX photoreactivation at pH 7 (A) and pH 4 (B). Vertical lines represent standard deviation.

3.3. Photoreactivation and dark repair

Photoreactivation and dark repair are possible side effects that may reduce the food quality and shelf-life of UV-C treated products. Figs. 3, 4 and 5 show the residual enzyme activity (LOX, POD and PPO) after UV-C treatment in buffer at pH 7.0 (A) and 4.0 (B) followed by storage for 24 h under light exposure and darkness. There were no significant differences ($p > 0.05$) between samples exposed to light and samples kept in darkness during storage for 24 h. This could be an indication that post-treatment light/dark exposure has no effect on repairing the UV damage. In general terms, there were also no significant differences ($p > 0.05$) between enzyme activities in samples just after the treatment and after the storage. However, there were cases where further activity decrease was noticed after storage in both light and dark exposure samples. For example, LOX activity further decreased during storage for all samples at pH 7.0 and for samples treated at 25 °C and pH 4.0. In other cases, enzyme activity increased after storage in both light and dark exposure samples [LOX activity increased by 50% after storage for the 65 °C-10 min sample at pH 4.0, POD activity increased by 28% for samples treated at 65 °C for 1 min at pH 7.0 and PPO activity increased by 25% for samples treated at 25 °C for 1 min at pH 4.0] suggesting that in these cases, enzymes were able to partially recover from the UV damage.

Several authors have observed the photoreactivation of *E. coli* cells (increase of colony forming units and repair of pyrimidine dimers) after treatment by UV-C and then exposure to fluorescent light but not after storage in darkness (Oguma et al., 2001; Sommer, Lhotsky, Haider, & Cabaj, 2000). Enzymes may undergo other mechanisms to repair the damage to their structures. For example, in some cases, enzymes are able to reconfigure their structure after processing (Anese et al., 1995; Gui et al., 2006; Schweiggert et al., 2006). In the present study, samples were stored for 24 h after processing. Whether longer storage can result in the recovery of enzyme activities is unknown.

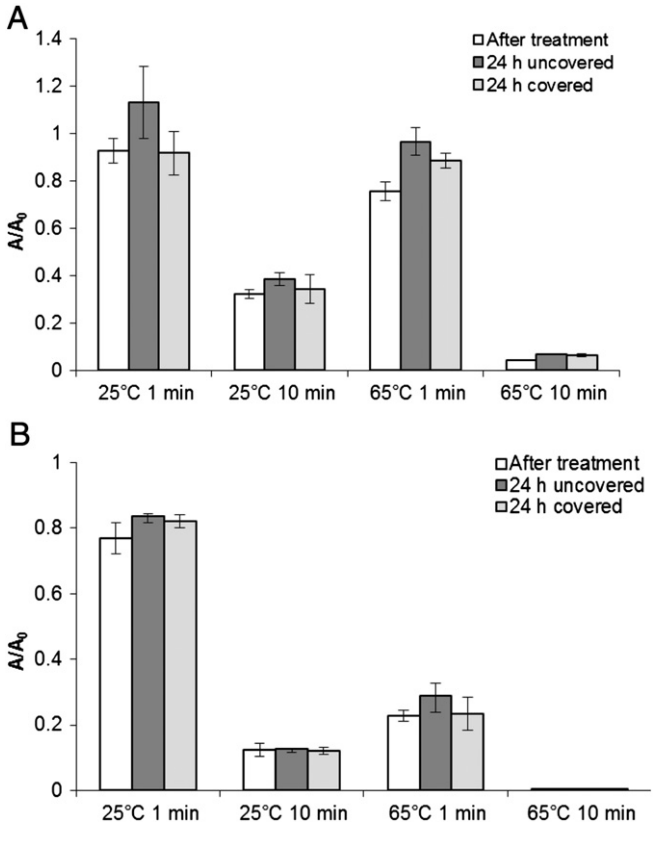

Fig. 4. POD photoreactivation at pH 7 (A) and pH 4 (B). Vertical lines represent standard deviation.

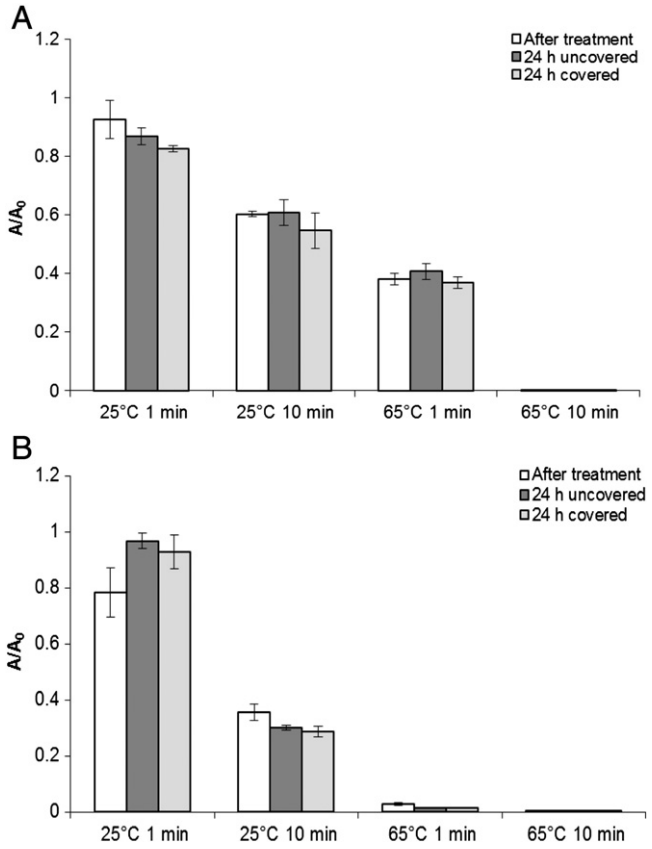

Fig. 5. PPO photoreactivation at pH 7 (A) and pH 4 (B). Vertical lines represent standard deviation.

4. Conclusions

The combination of temperature and UV-C was found to be an effective processing strategy to reduce the enzymatic activity in liquid foods. Deviations from the log-linear behavior were observed due to the appearance of upward and downward concavity responding to the multi-fraction nature of the enzymes. The Weibull model successfully characterized the combined thermal and UV-C inactivation kinetics of LOX, POD and PPO. Increased liquid absorbance was found to reduce the overall treatment effectiveness. Neither photoreactivation nor dark repair was observed during storage after the processing.

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References

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric field processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science & Emerging Technologies, 9*, 455–462.

Anese, M., Nicoli, M. C., Dall'Aglio, G., & Lerici, C. R. (1995). Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *Journal of Food Biochemistry, 18*, 285–293.

Anese, M., & Sovrano, S. (2006). Kinetics of thermal inactivation of tomato lipoxygenase. *Food Chemistry, 95*, 131–137.

Basaran, N., Quintero-Ramos, A., Moake, M. M., Churey, J. J., & Worobo, R. W. (2004). Influence of apple cultivars on inactivation of different strains of *Escherichia coli* O157:H7 in apple cider by UV irradiation. *Applied and Environmental Microbiology*, *70*(10), 6061–6065.

Cano, M., Hernandez, A., & DeAncos, B. (1997). High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *Journal of Food Science*, *62*(1), 85–88.

Elez-Martinez, P., Suarez-Recio, M., & Martin-Belloso, O. (2007). Modeling the reduction of pectin methyl esterase activity in orange juice by high intensity pulsed electric fields. *Journal of Food Engineering*, *78*, 184–193.

Espachs-Barroso, A., Barbosa-Cánovas, G. V., & Martín-Belloso, O. (2003). Microbial and enzymatic changes in fruit juice induced by high-intensity pulsed electric fields. *Food Reviews International*, *19*(3), 253–273.

Falguera, V., Pagan, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. *LWT—Food Science and Technology*, *44*(1), 115–119.

Garcia-Palazon, A., Suthanthangjai, W., Kajda, P., & Zabetakis, I. (2004). The effects of high hydrostatic pressure on beta-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria* × *ananassa*). *Food Chemistry*, *88*(1), 7–10.

Geveke, D. J. (2008). UV inactivation of *E. coli* in liquid egg white. *Food Bioprocess and Technology*, *1*, 201–206.

Guerrero-Beltran, J., & Barbosa-Canovas, G. (2004). Review: Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *10*(3), 137–147.

Guerrero-Beltran, J., & Barbosa-Canovas, G. (2006). Inactivation of *Saccharomyces cerevisiae* and polyphenoloxidase in mango nectar treated with UV light. *Journal of Food Protection*, *69*(2), 362–368.

Gui, F., Wu, J., Chen, F., Liao, X., Hu, X., Zhang, Z., et al. (2006). Change of polyphenol oxidase activity, color, and browning degree during storage of cloudy apple juice treated by supercritical carbon dioxide. *European Food Research and Technology*, *223*, 427–432.

Huang, K., Tian, H., Gai, L., & Wang, J. (2012). A review of kinetic models for inactivating microorganisms and enzymes by pulsed electric field processing. *Journal of Food Engineering*, *111*, 191–207.

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology*, *2*(2), 138–155.

Koutchma, T., Forney, L., & Moraru, C. (2009). Ultraviolet light in food technology: Principles and applications. *Ultraviolet light in food technology: Principles and applications*, 1–278.

Koutchma, T., Keller, S., Chirtel, S., & Parisi, B. (2004). Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Science and Emerging Technologies*, *5*, 179–189.

Lemos, M. A., Oliveira, J. C., Van Loey, A. M., & Hendrickx, M. E. (1999). Influence of pH and high pressure on the thermal inactivation kinetics of horseradish peroxidase. *Food Biotechnology*, *13*, 13–32.

Ludikhuyze, L., Van Loey, A., Indrawati, & Hendrickx, M. (2002). High pressure processing of fruit and vegetables. In W. M. F. Jongen (Ed.), *Fruit and vegetable processing: Improving quality*. Cambridge: Woodhead Publishing.

Ludikhuyze, L., Van Loey, A., Indrawati, Smout, C., & Hendrickx, M. (2003). Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: From kinetic information to process engineering aspects. *Critical reviews in food science and nutrition*, *43*(5), 527–586.

Matak, K. E., Churey, J. J., Worobo, R. W., Sumner, S. S., Hovingh, E., Hackney, C. R., et al. (2004). Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk. *Journal of Food Protection*, *68*(10), 2212–2216.

Morales-Blancas, E. F., Chandia, V. E., & Cisneros-Zevallos, L. (2002). Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. *Journal of Food Science*, *67*, 146–154.

Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple Juice. *Journal of Food Engineering*, *85*(1), 141–146.

Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T., & Ohgaki, S. (2001). Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. *Applied and Environmental Microbiology*, *67*(10), 4630–4637.

Oteiza, J. M., Peltzer, M., Gannuzzi, L., & Zaritzky, N. (2005). Antimicrobial efficacy of UV radiation on *Escherichia coli* O157:H7 (EDL 933) in fruit juices of different absorptivities. *Journal of Food Protection*, *68*(1), 49–58.

Quintero-Ramos, A., Churey, J. J., Hartman, P., Barnard, J., & Worobo, R. W. (2004). Modeling of *Escherichia coli* inactivation by UV irradiation at different pH values in apple cider. *Journal of Food Protection*, *67*(6), 1153–1156.

Rodrigo, D., Barbosa-Canovas, Gv, Martinez, A., & Rodrigo, M. (2003). Pectin methyl esterase and natural microflora of fresh mixed orange and carrot juice treated with pulsed electric fields. *Journal of Food Protection*, *66*, 2336–2342.

Rodrigo, D., Jolie, R., Van Loey, A., & Hendrickx, M. (2007). Thermal and high pressure stability of tomato lipoxygenase and hydroperoxide lyase. *Journal of Food Engineering*, *79*, 423–429.

Sampedro, F., Phillips, J., & Fan, X. (2013). Use of response surface methodology to study the combined effects of UV-C and thermal processing on vegetable oxidative enzymes. *LWT—Food Science and Technology*, http://dx.doi.org/10.1016/j.lwt.2013.07.010.

Sastry, S. K., Datta, A. K., & Worobo, R. W. (2000). Ultraviolet light. *Kinetic of microbial inactivation for alternative food processing technologies. Journal of food science supplement*. (pp. 90–92).

Schweiggert, U., Schieber, A., & Carle, R. (2006). Effects of blanching and storage on capsaicinoid stability and peroxidase activity of hot chili peppers (*Capsicum frutescens* L.). *Innovative Food Science & Emerging Technologies*, *7*(3), 217–224.

Sommer, R., Lhotsky, M., Haider, T., & Cabaj, A. (2000). UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. *Journal of Food Protection*, *63*(8), 1015–1020.

Tran, M. T. T., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science & Emerging Technologies*, *5*(4), 495–502.

U.S. Food and Drug Administration (2004). Code of Federal Regulations Title 21, Part 179, irradiation in the production, processing and handling of food.

Weemaes, C., Rubens, P., de Cordt, S., Ludikhuyze, L., Van den Brock, I., Hendrickx, M., et al. (1997). Temperature sensitivity and pressure resistance of mushroom polyphenoloxidase. *Journal of Food Science*, *62*(2), 261–266.